Ischemic Heart Disease

Use of Serum Enzyme Determinations in Diagnosis

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■ *The world literature since 1962 gives further substantiation of the usefulness of serologic measurement of glutamic-oxalacetic transaminase (GOT) activity and lactic dehydrogenase (LDH) activity in the diagnosis of myocardial infarction. LDH has not been found superior to GOT. The isoenzymes of LDH are now creating more and more interest because of their specificity for damage in various organs. This specificity, however, is not fully established.*

SO MANY AND SO RAPID are the developments in the use of enzymes in the diagnosis of ischemic heart disease that constant review is needed to keep abreast of the advancing knowledge. This review will cover only the aspects of serologic diagnosis of ischemic heart disease which relate to the world literature during the year 1963.

Serum enzymes which have found diagnostic application can be divided into two groups: In the first group are those enzymes which have a specific relationship to the tissue of origin and which have a high degree of specificity for a particular disease or group of diseases—for example, serum amylase or serum acid phosphatase. The enzymes in the second group are involved in important intermediary metabolic sequences and are found in many tissues in varying concentrations—the transaminases, for example. Damage to such tissues leads to the passage of these enzymes into the circulation regardless of the organ of origin but in different amounts for each enzyme present. The time sequence of release of a particular enzyme in a particular organ furnishes information which is becoming more and more valuable in the diagnosis of disease.

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White[18] pointed out some of the enigmas which still exist in the comparison of multiple serum enzyme levels. In studying patients with myocardial infarction he found a high serum lactic dehydrogenase (LDH) but a normal serum isocitric dehydrogenase (ICD) although the myocardium is rich in both enzymes. For elevations to occur, the equilibrium between the rate of enzyme elimination and the rate of entry from the blood must be disturbed; but there is no explanation of why certain enzymes do not increase when their tissue of origin is damaged. Such explanation must await better understanding of change of permeability of cells to enzyme content.

Wright and Warburton[70] stated that in 6 of 500 cases of acute myocardial infarction, glutamic-oxalacetic transaminase (GOT) and LDH were not elevated until 36 hours after onset. All these patients had very low blood pressures and the authors conjectured that hypotension may delay the rise. Sibley,[54] in a study of serum aldolase levels, pointed out that many questions concerning the basic physiology of serum enzymes still remain unanswered, among them the sources of abnormal enzymes in serum, how enzymes are eliminated from the blood, how normal levels are main-

tained, and the like. It has also been demonstrated that although excess liberation of an enzyme into the blood is usually the result of tissue injury, liberation may also occur without histological evidence of cellular destruction. Many other investigators are seeking to answer some of these questions.[8] Experimental myocardial infarction by several methods is being used to study the mechanism of enzyme liberation in injured tissue.*

In the diagnosis of myocardial infarction, many enzymes have been studied and reported to have value: glutamic-oxalacetic transaminase,† lactic dehydrogenase (LDH),‡ aldolase (ALD),[39,54] glutamic dehydrogenase (GDH), malic dehydrogenase (MDH),[62] hexose isomerase (HSI), succinic dehydrogenase (SUC), acid phosphatase (ACP), hexose kinase,[39] glucose 6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (ICDH), creatine phosphokinase (CPK),[10,14,21,27] and serum alpha-hydroxybutyric dehydrogenase (HBD).[13,27,31,47] Many other enzymes are also being reported as useful: free ribonucleotides,[26] tripeptidase,[46] serum phosphocreatine kinase,[18,49,55,56] and histamine.[59]

Accuracy of GOT and LDH

Of these GOT and LDH have been most useful. The literature has been summarized through 1962[1] with the following results: For GOT in 2,227 cases, accepting the clinical criteria for myocardial infarction, there was elevation in 2,148 cases, or 97 per cent correlation. In 142 autopsy cases GOT was elevated in 137. For LDH in 477 clinically proven cases there was correlation in 450 or 95 per cent.

Recently, Meyers and coworkers[35] reviewed 157 cases of myocardial infarction confirmed by autopsy and compared serum GOT activity rise with electrocardiographic alteration. Increased serum GOT activity was found in 97.3 per cent and diagnostic electrocardiographic changes in 81.5 per cent.

Significant rises in canine experimental pericarditis and pulmonary infarction has also been established.[1] Karmen and coworkers showed the higher GOT activity of human cardiac tissue as compared with the relatively lower activity of an equivalent weight of serum.[1]

Spicka and Kralova[57] investigated the GOT:GPT ratio in acute myocardial infarction. In 85 per cent of cases the ratio was higher than 1.5:1 but in 38 per cent of cases it was less than 2:1. The results indicated that a high ratio of 2:1 cannot always be expected even in uncomplicated cases and thus the ratio may have limited value in the differential diagnosis of myocardial infarction.

Lactic Dehydrogenase

As has already been mentioned, the serum LDH activity has also been found reliably elevated in the presence of myocardial infarction.[71] Usually, LDH activity appears and reaches its peak later than does GOT activity. It is particularly useful where the patient is seen late, since it tends to remain elevated several days longer than does GOT. But these temporal relations may vary. Opinions differ as to the relative value of the two enzyme tests. Some investigators have concluded (and others have agreed) that if LDH is not elevated, the diagnosis of myocardial infarction can be excluded. However, Wroblewski stated that LDH changes have not been found to be any more reliable or helpful than serum GOT alterations.[71]

Isoenzymes of LDH

Despite the usefulness of serum enzymes in diagnosing myocardial infarction, there are many situations in which the diagnosis may be confused because of their non-specificity. Thoracic pain in association with a rise in serum enzyme activity is not specific for myocardial infarction, with or without electrocardiographic changes, fever, leukocytosis or an increase in the sedimentation rate. All these changes may be seen in severe pericarditis, large pulmonary infarcts, myocarditis, dissecting aneurysm, pancreatitis, biliary tract disease, trauma to the chest and heart including surgical operations and strenuous physical exercise. Then there are enzyme changes in conditions which often accompany or complicate myocardial infarction. Such rises are found in congestive heart failure, shock, arrhythmias, infarctions of the kidney, diseases of the muscle, liver disorders, alcoholism, central nervous system diseases, tumors of the heart, neoplasms and the use of various drugs.

Such non-specificity promises to be lessened by the use of isoenzymes which have much higher

*Reference Nos. 15, 18, 24, 33, 37, 44.
†Reference Nos. 7, 9, 20, 25, 30, 32, 40, 41, 42, 60, 61, 63, 64.
‡Reference Nos. 5, 6, 11, 12, 23, 29, 34, 38, 43, 51, 58, 69, 71, 73.

organ specificity, particularly the isoenzymes of LDH.* This enzyme comprises five electrophoretically separable isoenzymes having the same substrate specificity but dissimilar mobility speeds due to differences in amino-acid composition. Techniques have been devised which permit separation of these isoenzymes by taking advantage of their different physical characteristics, such as heat stability. Human lactic dehydrogenase consists of five distinct components having a characteristic distribution.[71]

The fastest moving fraction lies between albumin and alpha$_1$ (LDH_5); the second on the fastest part of the alpha$_1$ (LDH_4); the third between beta$_1$ and beta$_2$ globulin (LDH_3); the fourth on the gamma-globulin fraction (LDH_2); and the fifth on the slowest part of the gamma-globulin (LDH_1). Tissues with a high aerobic metabolism, like cardiac muscle, have a high content of isoenzymes which move fastest in the electrophoretic field (LDH_4 and LDH_5). The slowest isoenzyme (LDH_1) is found in greatest concentration in the liver.†

Since heart muscle contains no LDH_1 and liver is poor in LDH_5, liver disorders can be distinguished from heart disorders by fractionation of the isoenzymes. The intermediately moving substances, LDH_2, LDH_3, and LDH_4 are usually highest in tumors. Zondag[73] confirmed these patterns in over 4,000 determinations.

Serum or plasma of humans contains all five isoenzymes, the concentrations diminishing in the order: LDH_4, LDH_5, LDH_3, LDH_2, and LDH_1. Samples should not be stored in the frozen state or LDH may be inactivated. Myocardial infarction is characterized by elevations of LDH_4 and LDH_5. LDH_5 rises higher than LDH_4. Such changes are claimed to be more specific of myocardial infarction than LDH unfractionated, GOT, ALD, ACP, MDH or SUC.

The importance of this field is attested by a symposium which was organized by the University of Ghent and the Belgian Society for Clinical Chemistry. It was called "Multiple Molecular Forms of Enzymes" and was held in Ghent in April of 1963. The purpose of the meeting was to explore the clinical uses of isoenzymes.[67] Evidence was adduced that isoenzymes like LDH_1 and LDH_5 are composed of at least four sub-units, and several methods of enzyme separation were discussed: electrophoresis and elution, such as cellulose acetate electrophoresis, the tetrazolium procedure, column chromatography, activity measured by 2-Ketobutyrate and heat separation, among other methods. Amelung[2] advocated the cellulose acetate absorption method. Wright and Warburton[70] recommended the chloroform method for differentiating cardiac isoenzymes from other serum lactic dehydrogenases. All the isoenzymes, apart from heart muscle LDH, are decidedly inhibited.

Vesell,[65] a pioneer in the field, warned against the specific diagnostic value of LDH isoenzyme tests, because patterns of similar character can be found in several disease states. Shock, in particular, may liberate LDH from many tissues and thus have a distorting effect.

In 43 patients with hypotension and the clinical features of shock caused by hemorrhage, infection or vascular obstruction and without evidence of cardiac or hepatic disease, elevation of GOT occurred in 70 per cent, LDH in 52 per cent, and GPT in 37 per cent. The presence of myocardial infarction under such circumstances would not be substantiated by such measurements.[52]

Dubach and coworkers[11,12] used the heat separation technique for the diagnosis of myocardial infarction and its distinction from pulmonary infarction where there is very little elevation of LDH_4 and LDH_5.

Batsakis[5] preferred two methods of separation: one which uses the heat-stable and heat-labile properties of the isoenzymes, and the other which utilizes their relative activities in catalyzing the reduction of pyruvate and alpha-oxobutyrate. The component that is active against alpha-oxobutyrate has been designated as "alpha-hydroxybutyrate dehydrogenase" (HBD), and its colorimetric measurement represents a chemical differentiation of the cardiac enzymes in serum. Elliott and Wilkinson[13] considered this to be the best test for the diagnosis of myocardial infarction. From observation of 200 cases Batsakis[5] concluded that HBD levels remain elevated longer than GOT and LDH (10 to 14 days) and will help greatly in distinguishing myocardial injury when other organ systems are also involved.

It appears that plasma or serum of patients with myocardial infarction, including subendocardial infarction, show changes in isoenzymes of LDH which are highly characteristic. However, the changes are not specific since similar changes have been observed in shock states, carcinoma of

*Reference Nos. 5, 11, 12, 58, 69, 71, 73.

†It must be borne in mind that in much of the literature the isoenzymes are numbered in the opposite direction, so that LDH_1 becomes LDH_5 and vice versa.

the stomach, hypothyroidism and pernicious anemia. These changes are more specific than any other enzyme measurements and are particularly useful when other organs are involved, especially liver disorders. Moreover, the rise in LDH_5 may occur as early as one hour after infarction and may last five to ten days longer than total enzyme elevation. Wroblewski[71] claimed that in subendocardial infarction LDH_5 may be elevated when GOT and other enzymes remain normal. The heat stability test is relatively simple whereas the electrophoretic techniques are tedious and require special equipment.

Other Enzyme Tests

Konttinen and Halonen[27] compared CPK and HBD activities with those of GOT and LDH in 34 clinically diagnosed myocardial infarctions. They found the most definite rise in HBD activity and this activity persisted longer than that of the other enzymes studied. CPK averaged only three days before returning to normal levels. Moreover, CPK determinations are technically more laborious. Measurement of CPK activity is useful in the appraisal of disease states complicated by liver involvement, since it does not rise in liver disease.

Many other enzyme alterations have been described in acute myocardial infarction. Nagano and Hochrein[39] have reported on aldolase, hexosekinase, glucose - 6 - phosphate - dehydrogenase (G6PDH), LDH, MDH, ICDH, and others, and have shown that all these enzyme systems are altered during insufficiency. No attempt is made to compare sensitivities. None of these many enzyme systems has been used widely enough to establish reliability and much more work is required to ascertain specificity. GOT particularly, and LDH, on the other hand have had wide study and application and their dependability is established. Also the degree of specificity and sources of error have been thoroughly investigated. It seems unlikely, therefore, that any of the more recently investigated systems will soon replace them in the diagnosis of myocardial infarction. At present isoenzymes carry the most promise for achieving greater specificity and of separating organ systems when the diagnosis is complicated by disease in several organs, for example liver and heart muscle.[16]

Several publications have reiterated that enzyme diagnosis is more sensitive than the electrocardiogram.[3] It has been well established that myocardial necrosis, confirmed by autopsy, can occur without electrocardiographic alteration in the presence of elevated enzyme activity.[17]

The value of enzyme elevation in separating minimal infarction from coronary insufficiency without myocardial necrosis is attested in recent studies.[4,39] Nevertheless, it must be repeated that a diagnosis of myocardial infarction cannot be established by increase in enzyme activity, even in association with pain, electrocardiographic changes, fever and leukocytosis, because such changes are not specific. They can be imitated by pericarditis, myocarditis, dissecting aneurysm, pancreatitis, biliary tract disease and the administration of some drugs.[53] On the other hand when enzyme activity is absent for 48 hours or more after the onset of persistent thoracic pain, then the possibility of infarction is so rare (less than 3 per cent) as eventually to rule out myocardial infarction.

In prognosis, it is still being found that the degree of infarction is roughly correlated with the height of the enzyme rise and thus with the mortality rate,[22,45] but such estimates are only a general guide. In the absence of shock, circulatory failure, or other source of enzyme liberation —from the liver, for example—high enzyme activity does have some relation to an increased mortality.

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